

### **ABSTRACT OF THE DISCLOSURE**

A method for quantitative determination of 25-hydroxycholecalciferol in feed is described. The method includes the steps of adding a defined amount of an internal standard which has a mass different from 25-hydroxycholecalciferol and a polarity similar to that compound, e.g., 26,27-hexadeutero-25-hydroxycholecalciferol, to an aqueous dispersion of the feed, extracting the aqueous dispersion with tert.butyl methyl ether and further processing the extract by HPLC and mass spectrometry as described in the specification.